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TECHNICAL MANUSCRIPT 313

MULTIPLE INFECTION OF CELL MONOLAYERS BY VIRUS MIXTURES

Nicholas Hahon

AUGUST 1966

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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MULTIPLE INFECTION OF CELL MONOLAYERS BY VIRUS MIXTURES

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Project 1C622401A071

August 1966

ABSTRACT

The applicability of selective immunofluorescent staining in conjunction with fluorescent cell-counting for the quantitative assay of virus mixtures is described. Multiple infection of cell monolayers was achieved with simultaneous inoculation by yellow fever, variola, and psittacosis agents in mixture. Each agent was assayed independently and no interference of viral growth occurred. Viral interference, manifested by the exclusion of yellow fever virus, was noted when the psittacosis agent and variola virus were inoculated 20 hours before the former.

MULTIPLE INFECTION OF CELL MONOLAYERS BY VIRUS MIXTURES

Mixed infections have assumed added importance in elucidating further the nature of viral control of intracellular activities with recent demonstrations of growth enhancement of one virus by another[1-3] and evidence of molecular interaction between unrelated viruses during their synthesis.[4-6] To facilitate experimental investigations of mixed infections at the cellular level, this communication describes conditions for inducing and detecting multiple infection of cell monolayers by virus mixtures and, in addition, describes a rapid and highly specific procedure for assaying quantitatively each viral agent in the mixture.

Each of the principal agents employed in this study had a different nucleic acid content, i.e., yellow fever (RNA), variola (DNA), and psittacosis (RNA and DNA). To effect multiple infection of cell monolayers, a mixture of the three agents in equal volumes and in an approximate virus-to-cell ratio of 1:50 was inoculated in 0.2-ml volumes onto cover slip cultures of McCoy cells. Centrifugation (19,000 to 29,000 x g, 15 min) was employed for adsorption of inoculum onto cover slip cell cultures held in rotor chamber inserts.[7] Cell cultures were then incubated at 35 C for 16 to 24 hours in accordance with previously established assays for each agent.[8,9] After fixing with acetone at -60 C, cover slip cell monolayers were separated into three groups and selectively stained with one of three viral-immune sera conjugated with fluorescein isothiocyanate. The infected cells were then counted. Results in Table 1 indicate that multiple infection of cell monolayers was achieved and that each agent in the mixture could be assayed independently. No enhancement or interference of growth occurred among the agents.

TABLE 1. MULTIPLE INFECTION OF McCOY CELL MONOLAYERS FOLLOWING INOCULATION WITH A MIXTURE OF THREE VIRAL AGENTS

Inoculum	Agent Assayed	Titer
		10^7 CIU[a]/ml
Mixture of yellow fever, variola, and psittacosis agents	Yellow fever	3.0
	Yellow fever + diluent[b]	2.8
	Psittacosis	6.4
	Psittacosis + diluent	6.2
	Variola	1.8
	Variola + diluent	1.4

a. Cell-infecting units; determined by selective immunofluorescent staining and fluorescent cell-counting.
b. Mixture 199 + 5% calf serum.

To show that a virus with a rapid growth rate may be assayed simultaneously with viruses of slower rates, Venezuelan equine encephalomyelitis (VEE) virus was incorporated as the fourth agent in the virus mixture. One hour after adsorption of inoculum, 1 ml of VEE hyperimmune serum was added to all cell monolayers. This prevented formation of VEE microplaques, which appear within 16 hours after inoculation, and the subsequent disorganization of cell monolayers by the virus. Primary VEE-infected cells could be counted then as early as 12 hours or as late as 24 hours.* The procedure for staining cell monolayers with conjugated immune serum was similar to that described earlier. The results indicated that the number of infected cells in cell monolayers inoculated with VEE virus alone was comparable to that of VEE virus in multiple-infected cell cultures. The presence of the fourth virus did not interfere with the growth of the other viruses.

Table 2 shows that selective immunofluorescent staining of virus-infected cell monolayers may be applicable to studies of virus interference.

TABLE 2. INOCULATION OF McCOY CELL MONOLAYERS WITH CHALLENGE VIRUS 20 HOURS AFTER INOCULATION OF VIRUS MIXTURE

Initial Agents	Challenge Agent	Growth of Agents		
		Yellow Fever	Psittacosis	Variola
Yellow fever and variola	Psittacosis	+[a]	+	+
Yellow fever and psittacosis	Variola	+	+	+
Psittacosis and variola	Yellow fever	Excluded	+	+

a. Number of fluorescent cells was comparable to that of control.

* [illegible] data.

When the psittacosis agent and variola virus were inoculated 20 hours before the addition of yellow fever virus, the latter was excluded. Subsequently, it was determined that the psittacosis agent alone could completely exclude yellow fever virus, but variola virus caused only a partial interference of growth. There was no reciprocity of interference. The phenomenon may be related to the presence of interferon, since it has been shown that yellow fever virus is 40 times more sensitive to interferon than variola virus or the psittacosis agent.* These preliminary experiments demonstrate the potential, versatility, and broad applicability of selective immunofluorescent staining in conjunction with fluorescent cell-counting for quantitative studies on multiple infection by virus mixtures.

* E.H. Kozikowski, unpublished data.

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